REMARKS

Claims 46, 49 and 53-63 were pending in this application. Claims 49 and 56-63 are now cancelled without prejudice to Applicants' right to prosecute their subject matter in the present application and in related applications. Claims 46 and 53-55 are amended without any intent of disclaiming equivalents thereof. New claims 64 and 65 are added. Accordingly, upon entry of this paper, claims 46, 53-55, 64 and 65 are pending and presented for consideration.

Claim amendments

Support for the amendments to claims 46, 53 and 55 and for new claims 64 and 65 can be found in specification as originally filed, for example, on page 20, lines 7-23.

Applicant respectfully submits that the amendments to the claims do not introduce new matter.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 54-63 are rejected under 35 U.S.C. § 112, second paragraph, for being indefinite. Specifically, the Examiner alleges that the phrase "the Factor V gene locus" cited in independent claim 54 lacks proper antecedent basis. Applicant has amended claim 54 to delete the rejected phrase. Accordingly, Applicant respectfully requests the rejection of independent claim 54 and its dependent claims be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph, scope of enablement

Claims 46, 49 and 53-63 stand rejected under 35 U.S.C. § 112, first paragraph, because, according to the Examiner, the specification does not enable any person skilled in the art to make the invention commensurate in scope with the claims without undue experimentation. Without acquiescing to the rejection, and solely to advance prosecution, Applicant has cancelled claims 49 and 56-63 without prejudice; the rejection with respect to claims 49 and 56-63 is therefore moot. Applicant traverses the rejection to the extent it is maintained over claims 46 and 53-55 as amended.

The test for enablement is whether one reasonably skilled in the art could make or use the invention as broadly as it is claimed based on the disclosures in the specification coupled with information known in the art without undue experimentation. See *In re* Wands, 858 F.2d 731

(CAFC 1988). In Wands, the court faced the question whether the specification of the Wands patent enabled one skilled in the art to make high affinity IgM monoclonal antibodies for hepatitis B-surface antigen. The Wands court recognized that the nature of monoclonal antibody technology involved screening hybridomas to determine which ones secrete antibodies with desired characteristics. Id. at 740. The court stated: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is 'undue,' not 'experimentation.'" Id. at 736-737. "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." Id. at 737. In deciding whether undue experimentation is involved for practicing the invention as claimed, the court considered the following eight factors: "(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability of the art, and (8) the breadth of the claims." Id. at 737. The court concluded that undue experimentation would not be required to practice the invention because (1) Wands' disclosure provided considerable direction and guidance on how to practice the invention, (2) there was a high level of skill in the art at the time when the application was filed, and (3) all of the methods needed to practice the invention were well known. Id. at 737, 740.

Applying the Wands analysis to the present application, Applicant submits the specification fully enables the invention as claimed in the claims as amended. Amended independent claim 46 relates to a method for detecting an individual at risk of developing thrombosis by detecting abnormal presence or absence of at least one nucleic acid fragment or sequence in the individual's Factor V gene compared to a normal control. Amended independent claim 54 relates to a method for determining a presence of a Factor V gene mutation associated with APC-resistance in an individual at risk of APC-resistance by determining the Factor V gene sequence obtained from the individual and comparing the sequence to a normal Factor V gene sequence. First of all, contrary to the Examiner's notion that the specification does not provide conclusive evidence of Factor V gene's involvement in thrombosis associated with APC-

resistance (see, Office Action, page 3), Applicant submits that the core of the present invention is the surprising discovery of a novel anticoagulant activity (*i.e.*, APC-cofactor activity) of Factor V and its involvement in thrombosis associated with APC-resistance. Throughout the specification, Applicant provides extensive clinical and biochemical evidence leading to the conclusion that Factor V has a novel anticoagulant activity and that deficiency of such activity causes thrombosis associated with APC-resistance. For example, as set forth on page 3, line 21, to page 4, line 15, the specification teaches the following:

> "According to the present invention it has been found that APC-resistance is due to deficiency of a previously unrecognized anticoagulant cofactor activity enhancing the proteolytic effect of APC directed against Factor Va and Factor VIIIa. . . . More specifically, this anticoagulant activity has been found to be expressed by Factor V, a finding that is quite surprising, since Factor V is the precursor to the procoagulant Factor Va, the latter being degraded by APC in the above mentioned Protein C anticoagulant system. . . . The discovery of the novel anticoagulant cofactor activity according to the present invention is based on the discovery of one patient with thrombosis and an abnormal APC-resistance when his plasma was assayed with the methods disclosed in WO 93/10261 (incorporated herein by reference) and by Dahlbäck et al. (Thromb. Haemost. 65, Abstract 39 (1991) 658). When studying a large cohort of patients with thrombosis, APC resistance was found to be the underlying cause in 30-40% of idiopathic thromboembolic events (Thromb. Haemost. 69, 999, abstract (1993))." [Emphasis added.]

On page 6, line 4, to page 7, line 12, the specification discloses 9 different categories of evidence supporting the conclusion that there exists a novel anticoagulant activity (*i.e.*, APC-cofactor activity) of Factor V. Copies of pages 6 and 7 of the specification listing 9 different categories of evidence are enclosed as Exhibit A for the Examiner's convenience. Furthermore, on page 20, lines 8-23, the specification discloses evidence establishing the molecular association between APC-resistance and the Factor V gene, *i.e.*, a strong linkage between a neutral polymorphism in the Factor V gene and the expression of APC-resistance. The Examiner stated in the Office Action: "Although we now know that the applicant was correct in asserting that a mutation in the Factor V gene is the cause of APC-resistance, see Bertina et al. [US 6,518,016 (2003)], at the time of the applicant's disclosure this was not know[n]. It is possible that the genetic defect leading to APC-resistance was the result of a defect in some other unknown gene physically close to the Factor V gene" (see, page 3 of the Office Action). However, in view of the extensive biochemical and clinical evidence corroborated by molecular

evidence disclosed in the present specification, one of skill in the art could not possibly have concluded that APC-resistance was caused by a defect in some other unknown gene physically close to the Factor V gene, as suggested by the Examiner.

Moreover, it was known in the art before the relevant priority date of the present application (July 20, 1993) that APC inactivates activated bovine Factor V by cleavage at least at position Arg505 which corresponds to Arg506 in human Factor V. Specifically, in 1987, Odegaard B. *et al.* determined that APC cleavage of the activated bovine Factor V heavy chain is responsible for the partial inactivation of the activated Factor V. Odegaard B. *et al.*, 1987, "Proteolysis of Factor Va by Factor Xa and Activated Protein C," J. Biol. Chem., 262:11233-11238, a copy of which is enclosed as Exhibit B. On June 30, 1993, Kalafatis M. *et al.* disclosed that "complete inactivation of [bovine] FVa by APC occurs after two cleavages of the FV_{aHC}: cleavage at Arg505 partially inactivates the cofactor, whereas coordinated cleavage at Arg505 and Arg306 is responsible for the complete inactivation of FVa." Kalafatis M. *et al.*, June 30, 1993, "Thrombosis and Haemostasis," page 759, Abstract 786, the XIVth Congress of the International Society on Thrombosis and Haemostasis, New York, U.S.A., a copy of which is enclosed as Exhibit C.

Therefore, based on the teachings of the present application and the knowledge in the art, one of skill would readily have concluded that a mutation in the Factor V gene is the most likely cause for thrombosis associated with APC-resistance.

Applicant further submits that, like in Wands, the specification of the present application provide reasonable guidance or directions on how to carry out the methods to detect a mutation in the Factor V gene as claimed in claims 46 and 54. For example, as set forth on page 20, lines 8-23, the specification teaches the following:

> Recent results have shown in a conventional DNA linkage study of a large family with inherited APC resistance that there is a strong linkage between a neutral polymorphism in the Factor V gene and expression of APC-resistance. This strongly suggests that a mutation in the Factor V gene is the cause for APC-resistance. This is conclusive evidence that nucleic acid hybridisation assays, as well as nucleic acid sequencing can be used in conventional ways in order to detect individuals at risk for thrombotic events due to a low level of APC cofactor 2 activity. Thus, these types of assays may be used for checking, in an individual, the abnormal presence or absence of one or more nucleic acid fragment(s) and/or

> sequence(s) unique for the presence or absence of expression of a Factor V molecule either carrying APC-cofactor 2 activity or being deficient in this activity. The protocols and conditions are the same as normally applied for other genes, except for now using reagents specific for the Factor V gene and, optionally, mutation(s) associated with APC-resistance or specific for a normal Factor V gene. Any cell sample from the individual may be appropriate.
>
> [Emphasis added.]

One of skill in the art upon review of the above paragraph would readily have understood how to carry out nucleic acid assays, such as hybridization or sequencing assays, using reagents specific to the Factor V gene in order to detect an individual at risk of developing thrombosis (*i.e.*, claim46) or to determine a presence of a Factor V gene mutation associated with APC-resistance in an individual at risk for APC-resistance (*i.e.*, claim 54).

Therefore, Applicant submits that the specification of the present application provides conclusive evidence of Factor V gene's involvement in thrombosis associated with APC-resistance and, like in Wands, provides reasonable guidance or directions on how to practice the invention as claimed in claims 46 and 54.

Secondly, Applicant submit that, like in Wands, where there was a high level of skill in the art of monoclonal antibody generation at the time when the application was filed, there was a high level of skill in the art of Factor V gene and protein when this application was filed. Specifically, both the Factor V protein sequence and the Factor V cDNA sequence were known in the art before the effective filing date of the present application. For example, Jenny *et al.* and Kane *et al.* reported complete cDNA and amino acid sequences of human Factor V in 1987. Jenny J.R. *et al.*, 1987, "Complete cDNA and derived amino acid sequence of human factor V," PNAS USA, 84:4846-4850, a copy of which is enclosed as Exhibit D. Kane W.H. *et al.*, 1987, "Cloning of a cDNA coding for human factor V, a blood coagulation factor homologous to factor VIII and ceruloplasmin," PNAS USA, 83:6800-6804, a copy of which is enclosed as Exhibit E. In addition, Cripe *et al.* reported the genomic structure of the human Factor V gene in 1992. Cripe L.D. *et al.*, 1992, "Structure of the Gene for Human Coagulation Factor V," Biochemistry, 31:3777-3785, a copy of which is enclosed as Exhibit F.

Furthermore, as discussed above, it was known in the art before the relevant priority date of the present application (July 20, 1993) that APC inactivates activated bovine Factor V by cleavage at least at position Arg505 which corresponds to Arg506 in human Factor V.

Therefore, Applicant submits that there was a high level of knowledge about the Factor V gene and protein in the art at the time when the application was filed allowing one of ordinary skill to determine possible mutations that could cause APC-resistance.

Thirdly, Applicant submits that, like in Wands, where methods needed to practice the invention were known in the art, all of the methods needed to detect abnormal nucleic acid sequence or to detect a presence of a mutation in the Factor V gene were well known in the art when the application was filed. In the art of molecular biology when this application was filed, it was routine for one of ordinary skill in the art to isolate nucleic acids from a cell sample, to conduct a nucleic acid assay such as hybridization or sequencing, to determine amino acid sequence and to compare the determined sequence to a known sequence in order to detect a presence of abnormal sequences. For example, general methods and tools were described in Alberts B. *et al.* (eds.), 1986, "Molecular Biology of the Cell," Garland Publishing, Inc., New York & London, pp 185-196, a copy of which is enclosed as Exhibit G. Since the publication of Alberts B. *et al.*, methods of sequencing had been further developed and became one of the most essential skills of ordinary artisan in the field of molecular biology at the time when the present application was filed. Methods and tools (*e.g.*, primers and other reagents) specific for the isolation, amplification and sequencing of the Factor V cDNA directly from human tissues, *e.g.*, lymphocytes, were disclosed in Shen *et al.*, April 1, 1993, "The Serine Protease Cofactor V Is Synthesized by Lymphocytes," J. Immunology, 150:2992-3001, a copy of which is enclosed as Exhibit H.

Furthermore, it was well within routine skills of an ordinary artisan to determine what amino acid substitution would be silent or cause only conservative substitution and what amino acid substitution would alter a protein's function. For example, similar or conservative amino acids in general were described in Dayoff *et al.*, 1978, Atlas of Protein Sequence and Structure, M. O. Dayoff, ed., Nat'l Biomed. Research Fnd., Washington D.C., vol. 5, Suppl. 3, pp. 345-362. With respect to Factor V, Shen *et al.* discussed in detail that there are certain mutations in the

Factor V gene that are silent or only cause conservative substitutions and may not significantly or only subtly affect the Factor V protein function. See, page 2996, left column of Exhibit H.

Thus, one of ordinary skill in the art would readily have been able to isolate and amplify nucleic acid from the lymphocytes of an individual who showed APC-resistance or was at risk of thrombosis associated with APC-resistance using specific methods and reagents described in Shen *et al.* (Exhibit H). One of skill would also readily have been able to sequence the nucleic acid using routine sequencing methods known in the art and reagents specific for the Factor V gene described in Shen *et al.* (Exhibit H). One of skill would readily have been able to determine abnormal presence or absence of nucleic acid sequence in the individual's Factor V gene associated with APC-resistance simply by comparing the sequence to a normal Factor V sequence disclosed by Jenny *et al.* (Exhibit D) and Kane *et al.* (Exhibit E). Then, one of skill would readily have been able to determine whether a particular mutation was associated with APC-resistance or was a silent mutation causing only conservative substitutions based on the common knowledge in the art and the teachings of Shen *et al.* (Exhibit H).

In fact, it was proven that a skilled artisan, based upon the disclosure of the present application and armed with the knowledge in the art, was able to determine the exact mutation in the Factor V gene that caused APC-resistance within a very short period of time. On June 18, 1994, Voorberg *et al.* reported that they determined a mutation at position Arg506 in the Factor V protein responsible for thromboembolism associated with APC-resistance based on the disclosure of the present application and the knowledge available in the art. J. Voorberg *et al.*, June 18, 1994, "Association of idiopathic venous with single point mutation at Arg506 of factor V," The Lancet, 343:1535-1536, a copy of which is enclosed as Exhibit I. The Arg506 position was known to be the site at which APC inactivates Factor Va as reported by Kalafatis M. *et al.* (Exhibit C). Thus, Voorberg's discovery was no surprising. It could have been readily identified by the skilled artisan as the cause of thromboembolism based on the disclosures of the present application. Voorberg *et al.* cited as reference 7 Dahlbäck B. *et al.*, February, 1994, "Inherited resistance to activated protein C is corrected by anticoagulant cofactor activity found to be a property of factor V," PNAS USA, 91:1396-1400, which summarizes the essential information of the present application and was published in February, 1994, soon after the present application

was filed; as reference 9 Kalafatis G. *et al.* which discloses the mechanism of APC inactivation of activated Factor V, an article corresponding to Exhibit C.

Specifically, Voorberg *et al.* analyzed 27 patients with documented idiopathic thromboembolism by APC-resistant test disclosed in the present application (see, Voorberg *et al.*, page 1535, left column, paragraph 3). Then, Voorberg *et al.* isolated RNA from peripheral blood lymphocytes as described by Shen *et al.* (Exhibit G) and prepared cDNA using techniques known in the art. The cDNA, especially the fragment containing the APC cleavage site Arg506, was amplified by PCR and sequenced using standard methods known in the art. The sequence was compared with the known cDNA sequence of a normal Factor V gene. The comparison showed that the patients having APC-resistance contained an abnormal sequence at position 506, a Gln instead of a normal Arg. Thus, Voorberg *et al.* determined the mutation in the Factor V gene responsible for APC-resistance. All of the experiments carried out by Voorberg *et al.* were either described in the present application or already known in the art and well within routine skills of an ordinary artisan. Therefore, none of the experiments were undue.

In summary, the present specification is sufficient to enable one of ordinary skill in the art to practice the claimed invention without undue experimentation because (1) the specification of the present invention provides sufficient support for a novel anticoagulant activity of Factor V, the deficiency of which causes thrombosis associated with APC-resistance, and adequate guidance or directions on how to carry out the claimed methods, (2) there was a high level of knowledge about the Factor V gene and protein at the time when the application was filed, and (3) all of the methods and tools needed to practice the invention were well known in the art.

Accordingly, Applicants respectfully request the rejection be reconsidered and withdrawn.

CONCLUSION

Applicant believes that all rejections have been overcome and all claims are in condition for allowance. The Examiner is invited to telephone the undersigned agent to discuss any remaining issues. Early and favorable actions are respectfully solicited.

Respectfully submitted,

Date: September 28, 2005
Reg. No. 51,551

Fangli Chen, Ph.D.
Agent for Applicants
Kirkpatrick & Lockhart Nicholson Graham LLP
75 State Street
Boston, Massachusetts 02109

Tel. No.: (617) 261-3198
Fax No.: (617) 261-3175
Customer Number: 022832